MR characterization of a syngeneic orthotopic ovarian tumor model

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Introduction

Epithelial ovarian cancer is the leading cause of death from gynecologic malignancy among women in developed countries, and accounts for about 15,000 deaths in the US annually. Worldwide, ovarian cancer is diagnosed in more than 225,000 women per year, leading to about 140,000 deaths. Although the prognosis in cases detected at an early stage is quite favorable, the vast majority of cases are diagnosed at an advanced stage when five-year survival rates are only 30-40%. The poor prognosis of ovarian cancer is due to a combination of the aggressive characteristics of the disease and a lack of effective therapy, further compounded by late detection and resistance of most relapsed tumors to current treatments. Metastases and malignant ascites are complications frequently observed in ovarian cancer at the time of diagnosis. Malignant ascites, a complication observed in terminal ovarian cancer, is a devastating condition that significantly contributes to poor quality of life and to mortality. This excess accumulation of fluid in the peritoneal cavity is due to a combination of impaired fluid drainage and to increased net filtration. Identifying mechanisms that drive the aggressiveness of ovarian cancers and its associated pathologies, such as the formation of metastases and the build-up of ascitic fluid, is urgently needed to provide new targets in effective control and treatment of ovarian cancer. Noninvasive magnetic resonance imaging (MRI) and magnetic resonance spectroscopic imaging (MRSI) provide opportunities to characterize the tumor microenvironment and to assess its relationship with ascites and metastases. In the present study, we applied MRI and MRSI to better understand the links existing between tumor vasculature, metabolism, and ascites build-up in an experimental model of ovarian cancer. We used a syngeneic orthotopic model of ovarian cancer where a piece of tumor tissue, derived from a subcutaneous tumor xenograft, is engrafted directly onto the ovary of immunocompetent female mice, to maintain the tumor physiological environment. The model frequently results in metastases and malignant ascites.

Methods

The ID8-VEGF-Defb29 cell line was used in the present study (1). We performed microsurgical orthotopic implantation of ovarian cancer tissue onto the ovary of C57BL6 mice. The tumor tissue pieces used for the implantation were obtained from subcutaneous tumors after inoculation of 2 x 10^6 cells in the flank of female C57BL6 mice. The mice implanted with orthotopic tumor were scanned every 2 weeks to assess tumor growth. Experiments were performed when the orthotopic tumors reached a size of 200 to 300 mm^3. In this orthotopic model, ascites, metastases in the peritoneal cavity, in the liver, on the diaphragm, and in distal lymph nodes were frequent, similar to human disease. The mice were scanned on a 9.4T spectrometer using a volume coil. T_1-weighted images were acquired to localize the tumors and measure their volume. ^{1}H MRSI and vascular MRI were performed using the macromolecular contrast agent albumin-gadolinium DTPA. Mice were then sacrificed, and the ascitic fluid volume was assessed. Mice were divided into 2 groups, low to no ascites (less than 1 mL), high ascites (more than 1 mL, up to more than 10 mL). Lungs, liver, and lymph nodes were excised and fixed in formalin to quantify metastatic spread.

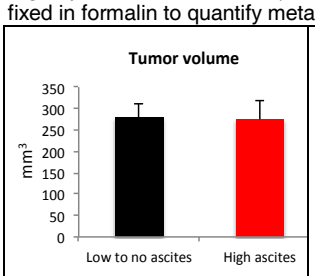

Figure 1: Tumor volume in mice with low to no ascites and in mice with high ascites (n=5)

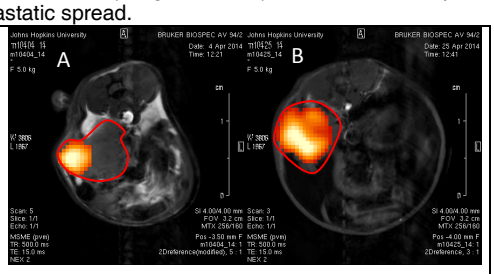

Figure 2: Representative tCho density maps in a mouse with no ascites (A) and in a mouse with high ascites (B). Tumors are highlighted in red.

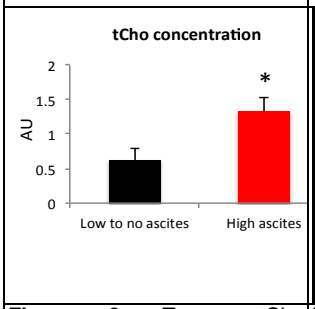

Figure 3: Tumor tCho concentrations in mice with low to no ascites and mice with high ascites (n=5 and n=7 respectively)

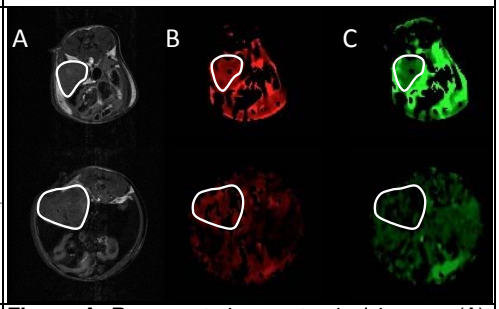

Figure 4: Representative anatomical images (A), vascular volume maps (B) and permeability surface area product maps (C) in a mouse with no ascites (top row) and in a mouse with high ascites (bottom row). Tumors are highlighted in white.

Results and Discussion

We observed that the presence of ascites was independent of tumor size (**Figure 1**). By scanning the mice when the tumors were about 200 to 300 mm^3, we were able to separate groups of mice with or without ascites. We observed a significantly higher concentration of total choline (tCho) in tumors from mice characterized by elevated level of ascites (**Figures 2 and 3**). Interestingly, we observed significantly lower vascular volume and lower permeability surface area product in the tumor from the mice with high volumes of ascites compared to the ones without ascites or with low levels (less than 1 mL) (**Figure 4**). These unexpected *in vivo* results might be due to the pressure created by the high volume of ascites in the peritoneal cavity that could lead to a collapse of the tumor vessel, blocking the delivery of the albumin-gadolinium-DTPA. We are currently analyzing tumor extracts with high-resolution ^{1}H MRS to better characterize the metabolic differences between both groups.

In the orthotopic model used in the present study, the occurrence of ascites was not related to tumor volume, and this model can be used to better understand the relationship between primary tumor characteristics and ascites formation. While permeability plays a critical role in ascites formation, our results showed that the pressure occurring from very high volume ascites may affect tumor vascularization. The intra-abdominal pressure created by the massive volume of ascites in the peritoneal cavity could be responsible for the decrease in vascular volume and permeability that we observed. In ovarian tumor patients, such a reduction could have major consequences for treatment delivery and therapeutic efficacy. The relationship between choline phospholipids metabolism and ascites formation needs to be further investigated.

References (1) Coukos *et al.,* Nature Medicine (2004).

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